

Support Vector Machines for Predicting Protein-Protein interactions using Domains and Hydrophobicity features

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Abstract—Since proteins work in the context of many other proteins and rarely work in isolation, it is highly important to study protein-protein interactions to understand proteins functions. The interactions data that have been identified by high-throughput technologies like the yeast two-hybrid system are known to yield many false positives. As a result, methods for computational prediction of protein-protein interactions based on sequence information are becoming increasingly important. In this study, computational prediction of protein-protein interactions (PPI) from domain structure and hydrophobicity properties, is presented. Protein domain structure and hydrophobicity properties are used separately as the sequence feature for the support vector machines (SVM) as a learning system. Both features achieved accuracy of about 80%. But domains structure had receiver operating characteristic (ROC) score of 0.8480 with running time of 34 seconds, while hydrophobicity had ROC score of 0.8159 with running time of 20,571 seconds (5.7 hours). These results indicate that protein-protein interaction can be predicted from domain structure with reliable accuracy and acceptable running time.

I. INTRODUCTION

A major challenge in bioinformatics is assigning function to newly discovered proteins. Although, most methods annotating protein function utilize sequence homology to proteins of experimentally known function, such a homology-based annotation transfer is problematic and limited in scope [1]. This is due to the fact that proteins work in the context of many other proteins and rarely work in isolation. The more we know about molecular biology the more we realize that protein-protein interactions affect almost all processes in a cell [2, 3]. For example structural proteins need to interact in order to shape organelles and the whole cell, molecular machines such as ribosomes or RNA polymerases are hold together by protein-protein interactions, and the same is true for multi-subunit channels or receptors in membranes. It is estimated that even simple single-celled organisms such as yeast have about 6000 proteins interact by at least 3 interactions per protein, i.e. a total of 20,000 interactions or more [4]. By extrapolation, there may be on the order of ~100,000 interactions in the human body.

As a result, identifying protein-protein interactions (PPI) represents a crucial step in understanding proteins functions. Most of the interactions data was identified by high-throughput technologies like the yeast two-hybrid system, which are known to yield many false positives [5]. In addition, *in vivo* experiments that identify protein-protein interaction are still time-consuming and labor-intensive; besides, they identify a small number of interactions. As a result, methods for computational prediction of protein-protein interactions based on sequence information are becoming increasingly important.

Over the past few years, several computational approaches to predict protein-protein interaction have been proposed. One of the earliest techniques was based on the assumption that protein-protein interactions are evolutionary conserved. It involves orthology-based mapping of a known reference interaction network to another, target organism [6].

Other methods relies on exploration of similarity of expression profiles to predict interacting proteins [7], coordination of occurrence of gene products in genomes, description of similarity of phylogenetic profiles [8] or trees [9], and studying the patterns of domain fusion [10]. However, it has been noted that these methods predict protein-protein interactions in a very general sense, meaning joint involvement in a certain biological process, and not necessarily actual physical interaction [11].

Another possibility to computationally predict interacting proteins is to correlate experimental data on interaction partners with computable or manually annotated features of protein sequences using machine learning approaches, such as support vector machines (SVM) [12] and data mining techniques, such as association rule mining [13].

The most common sequence feature used for this purpose is the protein domains structure. The motivation for this choice is that molecular interactions are typically mediated by a great variety of interaction domains [14]. It is thus logical to assume that the patterns of domain occurrence in interacting proteins provide useful information for training PPI prediction methods.

In a recent study, Kim et al. [15] introduced the notion of potentially interacting domain pair (PID) to describe domain pairs that occur in interacting proteins more frequently than would be expected by chance. Assuming that each protein in the training set may contain different combinations of multiple domains, the tendency of two proteins to interact is then calculated as a sum over log odd ratios over all possible domain pairs in the interacting proteins. Using cross-validation, the authors demonstrated 50% sensitivity and 98% specificity in reconstructing the training data set.

Gomez et al. [16] developed a probabilistic model to predict protein interactions in the context of regulatory networks. A biological network is represented as a directed graph with proteins as vertices and interactions as edges. A probability is assigned to every edge and non-edge, where the probability for each edge depends on how domains in two corresponding proteins “attract” and “repel” each other. The regulatory network is predicted as the one with the largest probability for its network topology. Using the database of interacting proteins, DIP [17], as the standard of truth and PFAM domains as sequence features, the authors built a probabilistic network of yeast interactions and reported very high true positive and true negative rates of 93 and 90%, respectively.

Another sequence feature that has been used to predict PPI *in-silico* is the hydrophobicity properties of the amino acid residues. Chung et al. [18] have used SVM learning system to recognize and predict PPI in yeast *Saccharomyces cerevisiae*. They selected only the hydrophobicity properties as sequence feature and combine it to the amino acid sequence of interacting proteins. According to their experiments, they reported 94% accuracy, 99% precision, and 90% recall in average. Although they achieved better results than the previous work using only hydrophobicity feature, their method of generating a negative dataset (i.e. non-interacting proteins pairs) is different from the previous work. They constructed the negative interaction set by replacing each value of the concatenated amino acid sequence with a random feature value. This approach may simplify the learning task and artificially raise classification accuracy for training data. There is no guarantee, however, that the generalized classification accuracy will not degrade if the predictor is presented with new, previously unseen data which are hard to classify. Therefore, in this study we proposed a better and more realistic method to construct the negative interaction set. Then we compared the using of domain structure and hydrophobicity properties as the protein features for the learning system. The choice of these two features is motivated by the previous discussed literature.

This paper is organized as follows. Section 2 gives a general description of our method to design feature space, select training data, and conduct learning. Section 3 describes protein interaction data sets used in this work as the standard of truth and the implementation of our predictor. In Section 4 we present and discuss experimental results of this work. Finally, some ideas on future directions are provided in Section 5.

II. METHODS

A. Support Vector Machines

The Support Vector Machine (SVM) is a binary classification algorithm. Hence, it is well suited for the task of discriminating between interacting and non-interacting protein pairs. The SVM is based on the idea of constructing the maximal margin hyperplane in the feature space [19]. Suppose we have a set of labeled training data $\{x_i, y_i\}$, $i = 1, \dots, n$, $y_i \in \{1, -1\}$, $x_i \in \mathbb{R}^d$, and have the separating hyperplane $(w \cdot x) + b = 0$, where feature vector: $x \in \mathbb{R}^d$, $w \in \mathbb{R}^d$ and $b \in \mathbb{R}$. In the linear separable case the SVM simply looks for the separating hyperplane that maximizes the margin by minimizing $\|w\|^2/2$ subject to the following constraint:

$$y_i(w \cdot x_i + b) \geq 1 \quad \forall i, i = 1, \dots, n \quad (1)$$

In the linear non-separable case, the optimal separating hyperplane can be found by introducing slack variables ξ_i , $i = 1, \dots, n$ and user-adjustable parameter C and then minimizing $\|w\|^2/2 + C \sum_i \xi_i$, subject to the following constraints:

$$y_i(w \cdot x_i + b) \geq 1 - \xi_i, \quad \xi_i \geq 0, \quad i = 1, \dots, n. \quad (2)$$

The dual optimization is solved here by introducing the Lagrange multipliers α_i for the non-separable case. Because linear function classes are not sufficient in many cases, we can substitute $\Phi(x_i)$ for each example x_i and use the kernel function $K(x_i, x_j)$ such that $\Phi(x_i) \cdot \Phi(x_j) = K(x_i, x_j)$. We thus get the following optimization problem:

$$\max_{\alpha} \sum_{i=1}^n \alpha_i - \frac{1}{2} \sum_{i,j=1}^n \alpha_i \alpha_j y_i y_j K(x_i, x_j) \quad (3)$$

$$\text{subject to } 0 \leq \alpha_i \leq C, \quad i = 1, \dots, n. \quad \& \quad \sum_{i=1}^n \alpha_i y_i = 0 \quad (4)$$

SVM has the following advantages to process biological data [11]: (1) SVM is computationally efficient and it is characterized by fast training which is essential for high-throughput screening of large protein datasets. (2) SVM is readily adaptable to new data, allowing for continuous model updates in parallel with the continuing growth of biological databases. (3) SVM provides a principled means to estimate generalization performance via an analytic upper bound on the generalization error. This means that a confidence level may be assigned to the prediction, and avoids problems with overfitting inherent in neural network function approximation.

B. Feature Representation

The initial step of any supervised learning process is the construction of an appropriate feature space to describe training examples. In the context of protein-protein interactions, it is believed that the likelihood of two proteins to interact with each other is associated with their structural domain composition [13, 14, 15]. It is also assumed that the hydrophobic effects drive protein-protein interactions [3, 18]. For these reasons, this study investigates the applicability of the domain structure and hydrophobicity properties as protein features to facilitate the prediction of protein-protein interactions using the support vector machines.

The domain data was retrieved from the PFAM database. PFAM is a reliable collection of multiple sequence alignments of protein families and profile hidden Markov models [19]. The current version 10.0 contains 6190 fully annotated PFAM-A families. PFAM-B provides additional PRODOM-generated alignments of sequence clusters in SWISSPROT and TrEMBL that are not modeled in PFAM-A.

When the domain information is used, the dimension size of the feature vector becomes the number of domains appeared in all the yeast proteins. The feature vector for each protein was thus formulated as:

$$x = [d_1, d_2, \dots, d_b, \dots, d_n] \quad (5)$$

where $d_i = m$ when the protein p has m pieces of domain d_i , and $d_i = 0$ otherwise. This formula allows the effect of multiple domains to be taken into account. Another representation is by using domain scores. In our case, each training example is a pair of interacting proteins (positive example) or a pair of proteins known or presumed not to interact (negative example).

In a similar approach, the amino acid hydrophobicity properties can be used to construct the feature vectors for SVM. The amino acids hydrophobicity properties are obtained from (Hopp & Woods, 1981). The hydrophobicity features can be represented in feature vector as:

$$x = [h_1, h_2, \dots, h_b, \dots, h_n] \quad (6)$$

where k is the number of amino acid in the protein x , $h_i = 1$ when the amino acid is hydrophobic and $h_i = 0$ when the amino acid is hydrophilic.

III. MATERIALS AND IMPLEMENTATION

A. Data sets

Protein interaction data can be obtained from the Database of Interacting Proteins (DIP; <http://www.dip.doe-mbi.ucla.edu/>). At the time of our experiments, the database comprises 15117 entries representing pairs of proteins known to mutually bind, giving rise to a specific biological function. Here, *interacting* mean that two amino acid chains were experimentally identified to bind to each other. Each interaction pair contains fields linking to other public protein databases, protein name identification and references to experimental literature underlying the interactions. Figure 1 shows a part of DIP, where each row represents a pair of interacting proteins (the third and the sixth columns represent proteins names).

DIP:2551N	AAC1	YMR055C	DIP:1103N	APG12	YBR217W	DIP:11374E
DIP:2551N	AAC1	YMR055C	DIP:1330N	LSM1	VJL124C	DIP:3267E
DIP:2551N	AAC1	YMR055C	DIP:4443N	PUF3	VLL013C	DIP:6745E
DIP:2551N	AAC1	YMR055C	DIP:2425N	RAO3	YER171W	DIP:13079E
DIP:6209N	AAC3	YBR035W	DIP:1103N	APG12	YBR217W	DIP:11375E
DIP:6209N	AAC3	YBR035W	DIP:5008N	BUD32	YGR252C	DIP:11546E
DIP:6209N	AAC3	YBR035W	DIP:1361N	HAP2	VGL237C	DIP:12245E
DIP:6209N	AAC3	YBR035W	DIP:963N	LAS17	VOR181W	DIP:12547E
DIP:6209N	AAC3	YBR035W	DIP:2425N	RAO3	YER171W	DIP:13080E
DIP:6209N	AAC3	YBR035W	DIP:2068N	RAO59	YOL059C	DIP:13131E

Fig. 1. A part of DIP database

The proteins sequences files were obtained for the Saccharomyces Genome Database (SGD; <http://www.yeastgenome.org/>). The SGD project collects information and maintains a database of the molecular biology of the yeast *Saccharomyces cerevisiae*. This database includes a variety of genomic and biological information and is maintained and updated by SGD curators. The SGD also maintains the *S. cerevisiae* Gene Name Registry, a complete list of all gene names used in *S. cerevisiae*. This task was transferred to the SGD by Dr. Robert Mortimer in early 1994. We have also compiled a set of general guidelines to gene naming that may be of help to researchers who are naming new *S. cerevisiae* genes.

The proteins sequence information is needed in this research in order to elucidate the domain structure of the proteins involved in the interaction and to represent the amino acid hydrophobicity in the feature vectors.

B. Data Preprocessing

Since proteins domains are highly informative for the protein-protein interaction, we used domain data as the main feature for protein sequence. We focused on domain data retrieved from the PFAM database, a reliable collection of multiple sequence alignments of protein families and profile hidden Markov models. In order to elucidate the PFAM domain structure in the yeast proteins, we first obtain all sequences of yeast proteins from SGD. Given that sequence file, we then run InterProScan [20] to examine which PFAM domains appear in each protein. We used the stand-

alone version of InterProScan. Apart from the result file is shown in Figure 2.

```
<?xml version="1.0" encoding="ISO-8859-1" ?>
<interpro_matches>
  <protein id="Q00845" length="594" crc64="5D555084C127E8B1">
    <interpro id="IPR000882" name="Cytochrome c oxidase, subunit I" type="Family">
      <child_list>
        <ref id="IPR004677" />
      </child_list>
      <match id="PF00115" name="COX1" dbname="PFAM">
        <location start="3" end="461" score="5.3e-302" status="I" evidence="HMMPFam" />
      </match>
    </interpro>
  </protein>
  <protein id="Q0050" length="834" crc64="BF86486F36416A5A">
    <interpro id="IPR000442" name="Intron maturase, type II" type="Family">
      <match id="PF01348" name="Intron_maturase2" dbname="PFAM">
        <location start="602" end="777" score="1e-75" status="I" evidence="HMMPFam" />
      </match>
    </interpro>
  <interpro id="IPR000477" name="RNA-directed DNA polymerase (Reverse transcriptase)" type="Domain">
    <ref id="IPR000123" />
    <ref id="IPR003286" />
    <ref id="IPR003545" />
    <found_in>
      <match id="PF00070" name="RT" dbname="PFAM">
        <location start="306" end="577" score="2.7e-77" status="I" evidence="HMMPFam" />
      </match>
    </found_in>
  </interpro>
</interpro_matches>
```

Fig. 2. A part from the protein domains file.

From the result file of InterProScan, we list up all PFAM domains that appear in yeast proteins and index them. Figure 3 shows an example of protein domains that appears in yeast genome. The first column represents a protein whereas the following columns represent the domains that appear in the protein. The order of this list is not important as long we keep it through the whole procedure. The number of all domains listed and indexed in this way is considered the dimension size of the feature vector, and the index of each PFAM domain within the list now indicates one of the elements in a feature vector.

proteins_domains.txt				
	YBL085W	PF00018	PF00169	PF07647 PF07653
	YBL087C	PF00238		
	YBL088C	PF00454	PF02259	PF02260
	YBL089W	PF01490		
	YBL091C	PF00557		
	YBL091C-A	PF00635		
	YBL092W	PF01655		
	YBL098W	PF01360		
	YBL099W	PF00006	PF00306	PF02874
	YBL101W-A	PF01021		
	YBL101W-B	PF01021	PF00665	
	YBL103C	PF00010		
	YBL105C	PF00168	PF00069	PF02185 PF00433 PF00130
	YBL111C	PF00270		
	YBR001C	PF01204	PF07492	

Fig. 3. An example of protein domains that appear in yeast genome.

The next step is to construct a feature vector for each protein. For example, if a protein has domain A and B which happened to be indexed 12 and 56 respectively in the above step, then we assign "1" to the 12th and 56th elements in the feature vector, and "0" to all the other elements. Next we focus on the protein pair to be used for SVM training and testing. The assembling of feature vector for each protein pair can be done by concatenating the feature vectors of proteins constructed in the previous step. Figure 5 shows the format of the feature vectors to be used by SVM.

When hydrophobicity is used, each amino acid will be replaced by 1 if it is hydrophobic and 0 if it is hydrophilic. Two separate training sets for domain and hydrophobicity features have been constructed.

IV. RESULTS AND DISCUSSION

In this study, we used the LIBSVM software [21] as a classification tool. The standard radial basis function (RBF) as available in LIBSVM was selected as a kernel function. Different values of γ for the kernel $K(x, y) = \exp(-\gamma \|x-y\|^2)$, $\gamma > 0$ were systematically tested to optimize the balance between sensitivity and specificity of the prediction. It is important to emphasize that in all our experiments we used only soft margin SVM. They are better suited for most real-world applications than hard margin SVM because the latter shows poor performance for overlapping classes; in our case, no priori knowledge was available on whether classes overlap or not.

Ten-fold cross-validation was utilized to obtain the training accuracy. The entire set of training pairs was split into 10 folds so that each fold contained approximately equal number of positive and negative pairs. Each trial involved selecting one fold as a test set, utilizing the remaining nine folds for training our model, and then applying the trained model to the test set.

The receiver operating characteristic (ROC) is also used to evaluate the results of our experiments. It is a graphical plot of the sensitivity (fraction of true positives - TP) vs. 1-specificity (the fraction of false positives - FP) for a binary classifier system as its discrimination threshold is varied. The sensitivity can be defined as: $TP / (TP + FN)$ where TP and FN stand for true positive and false negative, respectively. The specificity can be defined as: $TN / (TN + FP)$ where TN and FP stand for true negative and false positive, respectively. The area under the ROC curve is called ROC score.

In the table below, a comparison between domains structure and hydrophobicity as the protein feature is presented. The cross-validation accuracy results indicate that there is no significant difference when using domain structure or hydrophobic properties as the protein feature. However, ROC score indicates that domain structure is noticeably better than hydrophobic properties (Figure 4). Another aspect is the running time for both features. Evidently, when domain structure used, the data set is much smaller than the data set for the hydrophobic properties. Consequently, the running time required for domain structure training data is much less than the time running required for the hydrophobic training data as shown in Table I.

TABLE I
THE PERFORMANCE OF SVM FOR PREDICTING PPI USING DOMAIN AND HYDROPHOBICITY FEATURES.

Feature	Accuracy	ROC score	Running time
Domain	79.4372 %	0.8480	34 seconds
Hydrophobicity	78.6214 %	0.8159	20,571 seconds (5.7 hours)

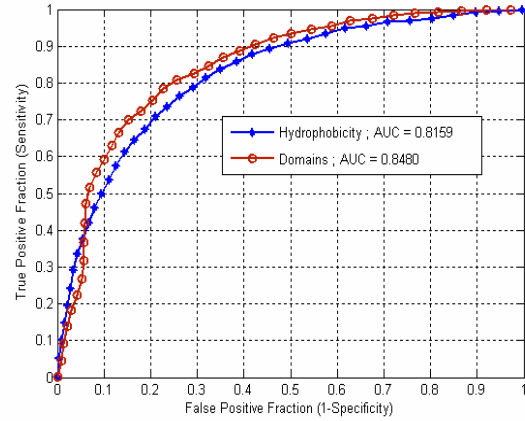


Fig. 4. The ROC plot for domain and hydrophobicity features.

V. CONCLUSION

The prediction approach reported in this paper generates a binary decision about potential protein-protein interactions based on the domain structure of the interacting proteins. One difficult challenge in this research is to find negative examples of interacting proteins, i.e., to find non-interacting protein pairs. For negative examples of SVM training and testing, we use a randomizing method. However, finding proper non-interacting protein pairs is important to ensure that prediction system reflects the real world. Discovering interacting protein patterns using primary structures of known protein interaction pairs may be subsequently enhanced by using other features such as secondary and tertiary structure in the learning machine. In conclusion the result of this study suggests that protein-protein interactions can be predicted from domain structure with reliable accuracy and acceptable running time. Consequently, these results show the possibility of proceeding directly from the automated identification of a cell's gene products to inference of the protein interaction pairs, facilitating protein function and cellular signaling pathway identification.

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